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OPTICAL DYNAMICS OF THE REACTION CENTER OF PHOTOSYSTEM II. A HOLE-BURNING AND PHOTON-ECHO STUDY

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Photon-echo and transient hole-burning experiments on the P-band of the reaction center of photosystem II are reported which show that the P-band exhibits a large homogeneous width. A photon-echo signal with a decay time of 500 ps is also observed in the same spectral region and assigned to extraneous chlorophyll. These observations are discussed with reference to the bacterial reaction center and photosystem I.

1. Introduction

With the recent elucidation of the spatial structure of the reaction center (RC) of the photosynthetic bacterium *Rhodopseudomonas* (*Rps.*) *viridis* by Deisenhofer et al. [1], spectroscopists have been greatly challenged to relate the spectroscopic observables of the RC to its structure. A remarkable feature of this pigment–protein complex, consisting of 4 bacteriochlorophyll (BChl)-**b** and 2 bacteriopheophytin (BPh) molecules is that it contains a pseudo twofold symmetry axis that passes through a co-facial BChl-**b** dimer, the so-called "special pair" P, which is at the heart of the RC. The charge-separation reaction however, is one-sided, the electron only passes along the L-branch of the pigment chain [2]. The apparent twofold symmetry therefore must be broken by pigment–protein contacts. The importance of these contacts to the optical dynamics of the RC is presently investigated by systematic study of genetically engineered mutants of a related photosynthetic bacterium, *Rps. capsulata* where selected proteins can be replaced in the structure [3]. While the gross features of the optical spectra of the reaction centers of *Rps. viridis* and *Rhodobacter* (*Rb.*) *sphaeroides* have been explained by Fischer and coworkers [4], understanding of the dynamics of the charge-separation reaction is still lacking. One particular intriguing riddle of the reaction center dynamics concerns the role of the accessory BChl molecule that is next to P. While its location between P and BPh strongly suggests it plays an important role in the electron-transfer process, femtosecond pump–probe experiments [5] have provided no evidence for this. In order to explain this observation several theories have been advanced [6]. Very recently it has been speculated [7] that a charge-transfer state of this accessory BChl molecule and the neighbouring BPh is located *below* the neutral dimer excitation and that energy transfer from P to this state presents the first step in the process of charge separation. In a second ultrafast step the dimer reduces the accessory $BChl^+$ thereby generating the famous radical pair P^+. Irrespective of whether this particular charge-transfer state is crucial to understanding of the photodynamics in the RC, the importance of charge-transfer states among the pigment molecules to functioning of the RC has been suggested earlier by Warshel [8] but remains a controversial subject. Recently it was suggested by Meech et al. on the basis of hole-burning [9,10] and photon-echo [9] experiments on the P-band of *Rps. viridis* and *Rb. sphaeroides* that an intra-dimer charge transfer state ($P^{\pm}$) plays an important role in the charge separa-

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tion reaction. This conclusion was based on the observation that the homogeneous width of the P-band in these reaction centers is extremely large compared to that of the Q(y) band of a monomer BChl molecule [9]. The large homogeneous width, in this picture, is due to strong vibronic coupling or mixing of the neutral Frenkel excitation with the charge-transfer state of the BChl dimer. In this vibronic mixing process, low-frequency modes associated with the relative motions of the BChl molecules in P are expected to be extremely important. The reason is that change of the dimer geometry strongly affects the Coulomb and exchange forces that determine the energetics of the special pair. In a dynamical picture, the large homogeneous width resulting from this mixing process could be interpreted, in terms of the zero-order Frenkel and intra-dimer charge-transfer state, as arising from an ultra-fast relaxation process; provided that the zero-order energy of the charge-transfer state is resonant or lower than the neutral BChl-dimer excitation. With the charge-transfer state at higher energy this picture is less appropriate, but the underlying physics, a congested level structure due to breakdown of the Born–Oppenheimer approximation, remains identical. Elaborate model calculations by Won and Friesner based on this idea [11] clearly show that such a vibronic mixing process provides an attractive interpretation of the observed large "homogeneous" width of the P-band. Moreover calculations by Warshel and Parson [12] show that one of the intra-dimer charge-transfer states is indeed expected to lie close to the neutral dimer excitation energy.

An alternative explanation of this large "homogeneous" width of the P-band was offered by Small and co-workers [13,14]. Their model assumes strong electron–phonon coupling of the P-band. It was also suggested that the phonons that play a role in this coupling process are those due to the protein–pigment contacts. The importance of charge transfer in the P-band was suggested by pointing at the similarity of electron–phonon coupling strength of the P-band with known charge-transfer states. Strong support for this model seemed to be provided by the observation of a sharp zero-phonon hole on top of a broad one in photosystem I [14]. Indeed observation of a zero-phonon line (or hole) in the P-band of reaction centers would be in conflict with the vibronic mixing model of Won and Friesner [11] and Meech et al. [9]. In view of the controversy regarding the interpretation of the large homogeneous width of the P-band and the implication hereof for the dynamics of the charge separation reaction, it seems important to provide more data on related photosynthetic systems.

In this Letter we report results of (transient) hole-burning and photon-echo experiments on the RC of PS II of green plants (spinach). An important feature of this RC is that its architecture is assumed to be similar to that of the purple photosynthetic bacteria [15]. In fact it was the homology of the amino acid sequence between the D1 and D2 subunits of PS II and the L and M subunits of the bacterial reaction center that led to the suggestion [5] that the D1–D2 cytochrome b-559 complex contained the RC of PS II. This suggestion has recently been confirmed by Nanba and Satoh [16]. Preparations based on their isolation procedure of the D1–D2 complex have led to the availability of a pigment–protein complex in which the ratio between Chl-a and Ph-a is 4.1:1 [17]. The complex, however, does not contain the secondary acceptor plastoquinone-9. In this paper we show that in this pigment–protein complex the P-band also exhibits a large homogeneous width (115 cm^{-1}), about a factor three less than in the cases of PS I [14] and the bacterial reaction center [9,10]. In a photon-echo experiment on the P-band of the RC, a long-lived state with a relaxation time of about 500 ps is detected. This state is assigned to chlorophyll molecules bound to the outside of the RC. Based on these observations it is suggested that the narrow hole earlier reported by Gillie et al. in PS I [14], is also due to hole-burning of extraneous chlorophyll and not of the RC.

2. Experimental methods

The population-bottleneck hole-burning and accumulated photon-echo experiments were basically performed as earlier described [9]. In the hole-burning experiments the exciting laser was modulated at about 20 Hz and the modulated transmitted probe beam phase-sensitively detected through a Spex 1402 monochromator by a photomultiplier (EMI 9816QB). Accumulated photon echoes were de-

tected using a double modulation technique as described by van Exter and Lagendijk [18]. In our apparatus standing wave modulator is used for high-frequency and a chopper for low-frequency modulation of the echo excitation and probe beams. The transmission measurements were made with a halogen lamp at 0.5 Å resolution (1 Å for the hole-burning experiments). Synchronous or tandem pumping of a dye laser was used to produce the appropriate radiation. In the hole-burning and photon-echo experiments, pyridine-1 in a propylenecarbonate/ethyleneglycol (1:5 vol/vol) solution was used as the lasing medium. For hole-burning experiments two etalons were added (with 30 GHz and 900 GHz free spectral range) to the dye-laser cavity. In the pump–probe experiments a dye laser, using the same lasing medium, was pumped by a synchronously pumped Na-fluorescein laser. The shortest clean pulses obtained were 1 ps. The RC (D1–D2 cytochrome b-559 complex) of PS II was isolated from spinach according to a procedure described by van Dorssen et al. [17]. Dilution of the samples was made with a glycerol/buffer (3:1 vol/vol) to give an OD$\leqslant$0.4 at 670 nm. Samples were degassed, sealed off and stored under liquid nitrogen; when used they were quickly cooled in a conduction cryostat to 77 K and then slowly to liquid helium temperature.

3. Results

3.1. Absorption spectroscopy

Fig. 1 shows the transmission spectrum in the Q(y) band region of the RC of PS II at 77 K and for comparison that of *Rb. sphaeroides* at 1.5 K. For the RC of PS II only a broad and slightly asymmetric band with a maximum at 670 nm is observed, in marked contrast to the highly structured spectrum of *Rb. sphaeroides*. This latter spectrum has been explained by Fischer and co-workers [4] using an excitonic model. In first order the band at 890 nm can be assigned to the asymmetric P-transition (P^a), the band at 800 nm to absorption of the accessory BChls and the 760 band to absorption by the BPhs. The symmetric P transition (P^s) is known to underlie the 800 nm band. Spectroscopic experiments on the D1–D2 complex by van Dorssen et al. [17] have shown that

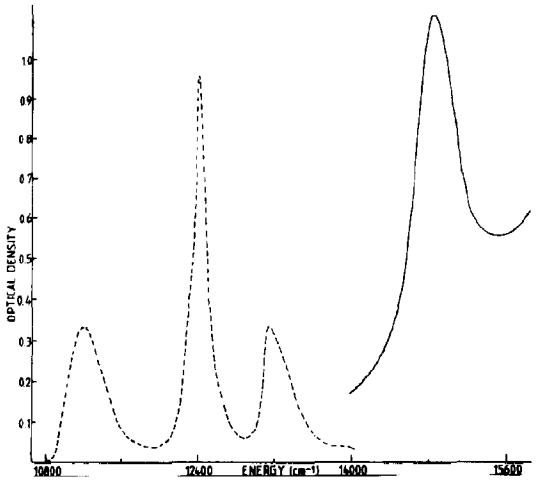

Fig. 1. Absorption spectra in the Q(y) region of the RC of *Rb. sphaeroides* at 1.5 K (dashed line) and the RC of PS II at 77 K (solid line).

the P^a transition in PS II is located at 682 nm, almost independent of temperature. The accessory Chls and Phs Q(y) absorptions have as yet not been identified but must underlie the broad band with a maximum at 670 nm. With a similar architecture of the plant and bacterial RCs, the lack of structure in the spectrum of RC of PS II in the Q(y) region is not expected to be due to a large reduction of the excitonic couplings but rather to site shifts caused by pigment–protein contacts. Only when the structure of the RC of PS II is known can this be definitely determined.

3.2. Hole-burning spectroscopy

Fig. 2 shows the hole-burning spectrum of the RC of PS II at 1.5 K obtained by taking the difference transmission spectrum with narrow-band laser excitation on and off. The exciting laser was modulated at about 20 Hz and had a width of 0.1 Å. A modulation depth of maximum 0.1 was achieved under optimal conditions with a fluence of 5 W cm^{-2}. The dash-dotted spectrum in fig. 2 is obtained with only the exciting laser beam present. This spectrum must be due to luminescence of the sample. As the fluorescence from P is expected to be considerably red-shifted, the resonant emission must be due to some other species, e.g. unconnected Chl. The dif-

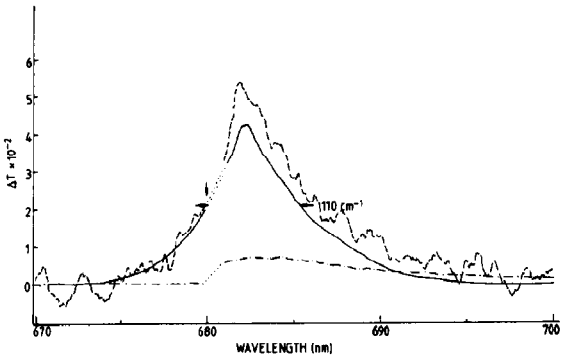

Fig. 2. Smoothed hole-burning spectrum of the RC of PS II (solid line) at 1.5 K. The noisy spectrum (dashed line) is the hole-burning spectrum uncorrected for the observed emission (dash-dotted line). In all experiments the laser wavelength was near 680 nm (vertical arrow in the figure). The dotted parts of the curves are interpolated and could not be directly recorded due to scattered laser light into the monochromator.

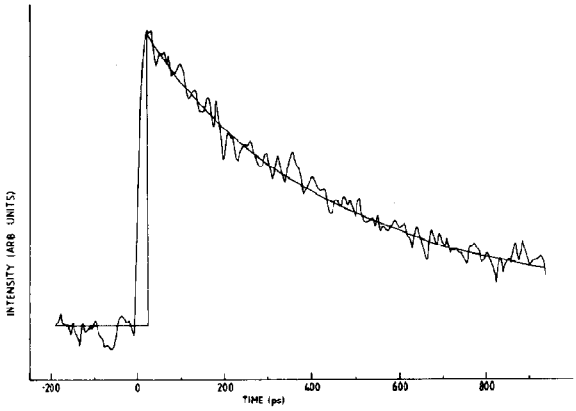

Fig. 3. Decay of the accumulated photon echo in the RC of PS II excited at 682 nm. The solid line is a double exponential fit with lifetimes of 217 ps (weight 0.3) and 698 ps (weight 0.7).

ficulty with this assignment is that the "luminescence" spectrum of the sample shows the same dependence on modulation frequency of the exciting laser as the hole-burning spectrum. This implies that both effects are dependent on population of the same bottleneck state. One interpretation is that the luminescence observed is delayed Chl fluorescence caused by annihilation of a triplet state in the RC and one from unconnected Chl. Irrespective of the precise interpretation of the luminescence spectrum, the true hole-burning spectrum is obtained by subtraction of the two signals and is presented as a solid line in fig. 2. As shown, the hole generated by the narrow-band excitation is very broad (115 ± 10 cm^{-1}). Wavelength-dependent hole-burning experiments further show that the hole width is little dependent on the excitation wavelength in the region from 678 to 685 nm. In addition when the excitation wavelength is changed the hole maximum shifts, showing that the inhomogeneous width of the P-band in the RC cannot exceed 200 cm^{-1}. When the sample temperature is raised the hole broadens to 190 cm^{-1} at 77 K. We note that the intensity of the hole-burning spectrum very quickly diminishes with an increase of modulation frequency over 20 Hz. This implies that the bottleneck state, probably a triplet spin sub-level state of P, must have a long-lived component that is efficiently populated by recombination of the radical pair state. No conclusion can be drawn from these experiments regarding the presence or absence of a zero-phonon line in the P-band. Scattered laser light prevents detection of such a feature in these transient hole-burning experiments and permanent hole-burning was not observed. This method therefore fails to prove or disprove the presence of a zero-phonon line (or hole) in the P-band of the RC of PS II. To try to come to a conclusion regarding this point we performed accumulated photon-echo experiments on the RC.

3.3. Accumulated photon-echo experiments

In a simple picture, the accumulated photon echo can be regarded as the time-domain analog of the transient hole-burning experiment. In both cases the "homogeneity" of a transition is probed on the time scale of the lifetime of the bottleneck. A broad hole therefore corresponds to a short and a narrow hole to a long component in the photon-echo decay. When both are present, the photon-echo decay will be non-exponential and exhibit both components in proportion to their intensity in the homogeneous absorption spectrum. Fig. 3 shows a typical example of a photon-echo decay obtained in PS II at 1.5 K. The signal around zero delay time is extremely strong and has been suppressed by the detection method. By comparing the intensities of both signals it is clear that the long decay component presents a weak signal with a lifetime of about 500 ps. The photon-echo decay in fact is non-exponential and can be fitted by a sum of two exponentials with time constants of 200

and 700 ps. This non-exponentiality we attribute to dispersion in the relaxation constants of Chl molecules involved. The signal, however, does not exhibit a maximum at 682 nm but is observed throughout the whole absorption band between 670 and 690 nm with little change in "lifetime". The intensity of the photon echo does not decrease with an increase of modulation frequencies up to a few hundred Hz, in contrast to the hole-burning signal. This leads to the conclusion that different bottleneck states are involved in hole-burning and photon-echo generation.

3.4. Time-resolved absorption experiments

We have also performed time-resolved absorption experiments on the P-band of the RC, in order to measure the electron transfer time from P to the neighbouring Ph molecule. Knowledge of this transfer time is important as it presents an upper limit for the long component in the photon-echo decay at low temperature. In bacterial reaction centers this transfer time is about 2.8 ps at room temperature and about a factor of four faster at 4 K [23]. Danielius et al. [19] have shown that in PS II this transfer time at room temperature is less than 25 ps. The pump–probe experiments were performed with the same apparatus as for photon-echo detection, except for insertion of an acousto-optic modulator, which is known to introduce phase jitter between consecutive pulses of the exciting pulse train. This is important for discrimination between accumulated coherent and incoherent effects at these high repetition rates (92 MHz) [20]. The pump–probe experiments were performed using excitation and probe pulses of 1 ps; however, no transient signal outside the pulse correlation width is observed. One is therefore tempted to conclude that the electron-transfer time is less than 1 ps. However, as we have only observed such signals for parallel polarization and not for perpendicular polarization of the pump and probe pulses we consider our experiment inconclusive. We note that with the same apparatus we observed large transient signals in the B850-800 complex for excitation in the 850 nm band. Experiments with amplified picosecond pulses are being planned to confirm the ultrafast nature of the electron-transfer process in the RC of PS II.

4. Discussion

The results of this study show that in the RC of PS II as in bacterial reaction centers, the P-band is largely homogeneous. Whether this "homogeneity" is due to vibronic mixing or electron–phonon coupling remains the question. The answer is important as it may relate to the more general dilemma of why in photosynthetic reaction centers a cofacial dimer acts as the primary donor in the electron-transfer process. This in turn relates to the question whether or not charge-transfer states play an important role. In the vibronic mixing model the P-band has acquired charge-transfer character by mixing a neutral (Frenkel) dimer transition with a nearby charge-transfer state. This model then leads to the prediction of a large dipole-moment change in the P-band, in agreement with observation [21,22]. In the electron–phonon coupling model the deduced strong electron–phonon coupling is taken as evidence for charge-transfer character of the P-band. The crucial factor however, in discriminating directly between the predictions of the two models is the presence or absence of a zero-phonon line (ZPL) in the P-band. If such a transition is present, the electron–phonon coupling description of the P-band is appropriate. If not, the case is undecided, unless the electron–phonon coupling model would lead to internal inconsistencies with regards to the calculated Huang–Rhys (S) factor from the Stokes shift and the value of S needed to fit the absorption width. S is a measure for the electron–phonon coupling strength and the intensity of the ZPL with respect to the total intensity is given by $\exp(-S)$. The homogeneous width of the ZPL ($\Delta\nu_h$) is related to the photon-echo decay time (T_2) by the relation $\Delta\nu_h = 1/\pi T_2$ whereby at 0 K the relation $T_2 = 2T_1$ holds. Here T_1 would be the transfer time of the electron (or excitation [7]) from P to Ph (or $Chl^+ \cdot Ph^-$ [7]). Assuming then that the observed echo decay of 500 ps in RC of PS II arises from the sharp (0.01 cm^{-1}) zero-phonon line of P one would have to conclude that the electron-transfer time exceeds 500 ps. This conclusion seems in conflict with a reported upper limit of 25 ps at room temperature for this constant by Danielius et al. [19]. In view of recent findings on the temperature dependence of this transfer time in *Rps. viridis* [23] it seems unlikely that at low temperature the transfer

rate is reduced by a factor of more than 20. We therefore conclude that the long component in the echo decay is due to Chl unconnected to the reaction center. The lifetime of the photon echo is most likely determined by dephasing processes, which in amorphous hosts are known to occur on this time scale [24]. Two conclusions can be drawn: the first is that the photon-echo experiments do not provide evidence for a ZPL in the P-band of the RC of PS II. We add however that these experiments do not prove its absence either; the echo amplitude due to the ZPL may simply be below our detection sensitivity. The second conclusion is that the electron-transfer process in PS II is on the picosecond time scale. Our experiments also show that in the presently available plant photosystems, unconnected chlorophyll can have marked effects on the spectroscopic observables. We therefore suggest that the sharp hole reported by Gillie et al. [14] is due to unconnected chlorophyll and is not part of the hole-burning spectrum of the RC. Further experiments on the RC of PS II are called for to put a more stringent test on the applicability of existing theories to describing the large "homogeneous" width of the P-band in RCs.

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